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13. ABSTRACT (Maximum 200 Words)

Our previous work demonstrated that prostate cancers differ from benign prostatic epithelium by expressing oncogenic members of the pp32 gene family. Whereas benign prostatic epithelium solely expresses pp32, prostate cancers express pp32r1 and pp32r2, which are oncogenic. The purpose of the study was to confirm and extend these preliminary results, to develop practical means to assay pp32 gene family members in clinical samples, and to determine the clinical significance of their presence. The approved proposal encompassed four broad tasks: [1] characterization of the pp32 expression phenotype of a larger sample of 40 prostatic adenocarcinomas; [2] development of a practical molecular pathology assay for altered pp32 transcripts; [3] adaptation of the assay to paraffin-embedded tissue; and [4] preliminary determination of the clinical utility of pp32r1 and pp32r2 expression in prostatic adenocarcinoma. In the course of developing these previously reported assays, a mutation in pp32r1 was detected in a human prostatic cancer cell line in pp32r1 involving a T to C transversion at position 418; transfection studies showed this to cause increased cell proliferation. A PCR assay is now being used to determine the frequency of the pp32r1 mutation in prostate cancers. Antibodies will be used to examine pp32 gene family expression in prostate cancers.

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INTRODUCTION

Since the last report, further progress in the fundamental biology of pp32 has underscored its prominence as a key regulator of important cellular processes:

Reduction of pp32 by antisense or siRNA induces cellular differentiation with discrete associated changes in gene expression profiles (1)

pp32 is a key participant in granzyme A-mediated apoptotic pathways (2,3); it also participates in a mitochondrial pathway regulating caspase activation (4).

pp32 regulates gene expression as part of a complex that inhibits histone acetylation (5)

pp32 is a regulator of mRNA stability and trafficking (6-8)

Both prostate and breast cancers express pp32r1 and pp32r2, whereas normal epithelium expresses predominantly pp32 (9,10)

Taken together, these findings strongly indicate that pp32 is involved in control of the critical programmatic decision that cells make as to whether to retain the capacity to proliferate, or whether to undergo differentiation.

The approved proposal encompassed four technical objectives: [1] characterization of the pp32 expression phenotype of a larger sample of 40 prostatic adenocarcinomas; [2] development of a practical molecular pathology assay for altered pp32 transcripts; [3] adaptation of the assay to paraffin-embedded tissue; and [4] preliminary determination of the clinical utility of pp32r1 and pp32r2 expression in prostatic adenocarcinoma.

BODY

Task 1. This approved task involves characterization of abnormal pp32 transcripts in frozen samples of human prostatic adenocarcinoma compared to paired normal prostate controls. 40 pairs of prostatic adenocarcinoma and normal prostate are to be analyzed to determine the range and frequency of occurrence of pp32 gene family-related sequences in prostatic adenocarcinoma.

Progress: Screening of a human prostatic adenocarcinoma cell line identified a T to C transversion mutation at position 418 that results in a change from tyrosine to histidine at amino acid 140. The change, along with polymorphisms, is shown in Table I:

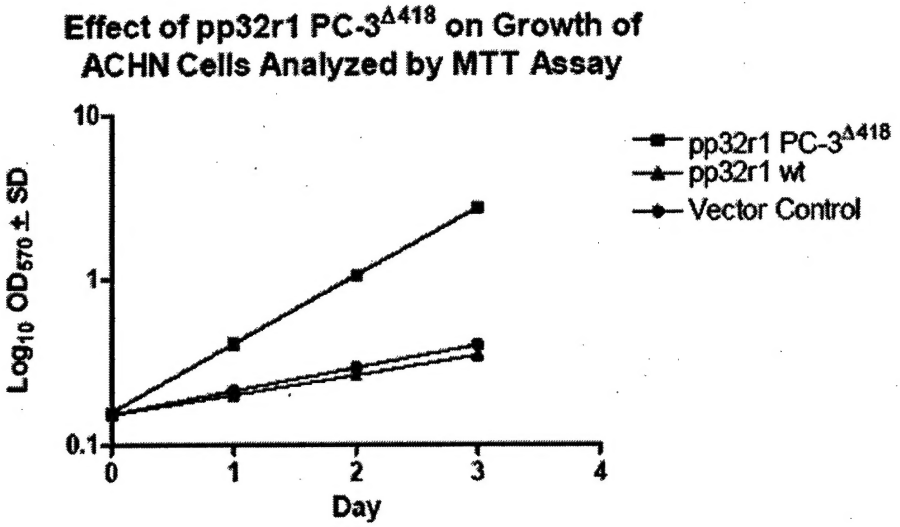
Table 1

Genomic DNA Source	Nucleotide Changes	Amino Acid Changes
Normal Human Placenta	None	None
Tobacco Associated Oral Mucosal Lesions #1 - #7	None	None
Tobacco Associated Oral Mucosal Lesion #8 - #9	g.68C>T, g.212G>A	23A>V, 71R>K
Normal, Human Fibroblastic Cell Lines CCD-27SK, CCD-32SK, CCD-42SK, and CCD45-SK	g.68C>T, g.212G>A	23A>V, 71R>K
Normal, Human Fibroblastic Cell Lines CCD-34SK, CCD39-SK, and CCD-965SK	g.212G>A	71R>K
Human Carcinoma Cell Lines ACHN, BT20, CALU-3, and DU 145	g.68C>T, g.212G>A	23A>V, 71R>K
Human Prostatic Adenocarcinoma Cell Line PC-3	g.68C>T, g.212G>A ,g.418T>C	23A>V, 71R>K, 140Y>H

The reference sequence is AF008216.1 GI:2738512.

When transfected into ACHN cells, the prostatic adenocarcinoma cell line mutation caused a significant increase in cell growth, as determined by the MTT assay that measures cell metabolic activity, as seen in Figure 1.

Figure 1



This work is currently in press in Human Mutation.

Task 2. This approved task aims at development of a practical molecular pathology assay to distinguish individual members of the pp32 gene family. Briefly, this task involves selection and optimization of PCR primer sets for efficient amplification of altered regions of pp32. The original objective was to select and optimize restriction enzyme cleavages to distinguish among normal pp32 and the various altered forms of pp32. This involved standardization of the assay using defined mixtures of plasmid DNA to determine sensitivity and specificity under optimized PCR conditions and comparison of assay performance on known samples of RNA from frozen tissue.

Progress: Very recently, antibodies that distinguish pp32, pp32r1, and pp32r2 became available to this project (See final report for DAMD-17-99-9240). pp32, pp32r1, and pp32r2 each possess a unique pattern of reactivity that permits them to be distinguished (see Table 2, below, reproduced from the previously cited report). The previously developed molecular assays proved to be too cumbersome for routine use. It is anticipated that standard immunohistochemical techniques will now make this portion of the study straightforward in work that is anticipated to continue beyond the project period.

Table 2. Summary of Antibody Reactivities

Protein	Antibody			
	anti-pp32 GRP	anti-pp32 MARL	anti-pp32r1 MARL	anti-pp32r2 MARL
pp32	+	+	-	-
pp32r1	+	-	+	+
pp32r2	-	-	+	+

Task 3. This approved task aims to adapt the molecular pathology assay for use archival tissue. Briefly, the task involves preparation of RNA from set of paraffin-embedded human prostatic adenocarcinomas, paired adjacent normal prostates, and control tissues. This is followed by amplification of pp32 mRNA by RT-PCR and analysis by the assay developed in Task 2, and validation of the assay by subcloning and sequencing of selected regions or entire inserts as indicated, using methods described for Task 1.

Progress: As described under Task 3, the antibodies that are now available will be adapted to use in evaluation of prostate cancer specimens using routine immunohistochemical techniques.

Task 4. This approved task seeks a preliminary determination of the clinical significance of pp32 molecular changes. This task requires assembly of paraffin blocks from previously studied population of prostatectomy specimens (1), preparation of RNA from paraffin sections, analysis of pp32 RNA by molecular assay developed under Tasks 2 and 3. The results will be selectively validated by selective subcloning and sequencing, as described in Task 1.

Progress: Work on this task remains scheduled to begin as soon as Task 3 is complete. Work will continue on this project beyond the funding period in order to accomplish the approved tasks despite the frustrating delays introduced by unanticipated and thorny problems in assay development. The clinical goals of the project were, and remain, valid.

KEY RESEARCH ACCOMPLISHMENTS

While practical molecular assays remained elusive, antibodies that recently became available that will permit routine analysis of pp32 gene family member expression.

The assay is applicable to paraffin sections and will shortly be validated (Task 3).

A functional mutation of pp32r1 was discovered; its significance in prostate cancer will be determined in work beyond the scope of this project.

REPORTABLE OUTCOMES

Kochevar J.G., Brody J.R., Kadkol S.S., Murphy K.M., and Pasternack G.R. Identification of a Functional Mutation in pp32r1 (ANP32C). 2004. Human Mutation, In press.

CONCLUSIONS

Quantitative analysis of pp32 gene family members in human prostate cancer and benign tissues is now finally ready to be applied to clinical specimens using immunohistochemistry rather than molecular assays as originally intended. The results are expected to be particularly informative since recent data, discussed in the Introduction, makes pp32 an interesting and potentially very important analyte.

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APPENDICES

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Moushira Mahmoud MBBS	Post-Doctoral Fellow
Bruce Huang	Technician

Publications & Abstracts during Project Period Including No-Cost Extension

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